Food and Agriculture Organization of the United Nations

World Health Organization

Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 92nd Meeting 2021

β-glucanase from *Streptomyces violaceoruber*

This monograph was also published in: Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 92nd meeting 2021. FAO JECFA Monographs 27

β - GLUCANASE FROM *STREPTOMYCES VIOLACEORUBER*

New specifications were prepared at the 92nd JECFA (2021), published in FAO JECFA Monographs 27 (2021). An ADI of "not specified" was established at the 92nd JECFA (2021).

SYNONYMS endo-1,3-β-glucanase; laminarinase; laminaranase; oligo-1,3-glucosidase; endo-1,3-β-glucanase; callase; β-1,3-glucanase; kitalase; 1,3-β-D-glucan 3-glucanohydrolase; endo-(1,3)-β-D-glucanase; (1→3)-β-glucan 3-glucanohydrolase; endo-1,3-β-D-glucanase; endo-1,3-β-glucosidase; 1,3-β-D-glucan glucanohydrolase

SOURCES Produced by controlled fed-batch fermentation of a non-pathogenic, non-toxigenic strain of *Streptomyces violaceoruber*. The secreted β-glucanase is separated from the biomass and concentrated using sedimentation followed by a series of filtration steps. The β-glucanase concentrate may be formulated into either a liquid or a powder enzyme preparation using food-grade stabilizing and preserving agents.

Active principles Glucan endo-1,3-β-D-glucosidase

Systematic names and numbers 3-β-D-Glucan glucanohydrolase; EC 3.2.1.39;
CAS No. 9025-37-0

Reaction catalysed Hydrolysis of the (1->3)- β-D-glucosidic linkages in (1->3)- β-D-glucans to produce corresponding β-glucans and glucose

Secondary enzyme activities No significant levels of secondary activities

DESCRIPTION Brown, liquid or powder

FUNCTIONAL USES Enzyme preparation
Used in the manufacture of beer, yeast and mushroom extracts.

GENERAL SPECIFICATIONS Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION

β-Glucanase activity The sample shows beta-glucanase activity.
See description under TESTS.

TESTS

METHOD OF ASSAY

β-Glucanase activity

Principle

Assay measures the amount of glucose produced by spectrophotometry using the phenol-sulfuric acid method when curdlan is treated with β-glucanase.

One unit of activity is defined as the quantity of beta-glucanase required to produce 1µmol of D-glucose per minute under the conditions of the assay.

Apparatus

Water bath with circulation
UV-Vis spectrophotometer
pH meter
Vortex mixer

Reagents and Solutions

- 0.5 M Hydrochloric acid: Prepare by mixing 9 ml of 6.0 M hydrochloric acid with 99 ml of deionised water.
- Phenol solution (5%; w/v): Dilute 25 g of phenol (anhydrous), adjusted for purity, to 500 ml with deionised water.
- 1 M Acetic acid: Prepare by adding deionised water to 60 g of acetic acid and fill to 1 litre using a measuring flask.
- 1 M Sodium Acetate solution: Prepare by adding distilled water to 82 g of anhydrous sodium acetate and fill to 1 litre in a measuring flask.
- 1 M Acetate buffer (pH 5.0): Prepare by mixing 1 M acetic acid and 1 M sodium acetate and adjust the pH to 5.0.
- Enzyme diluent solution: Dilute 1 M Acetate buffer (pH 5.0) ten-fold in deionised water.
- D-Glucose stock solution: Transfer 0.901 g D-glucose to a 500 ml volumetric flask; dissolve in and dilute to volume with deionised water.

Substrate solution

Dilute 3 g of curdlan (Fujifilm Wako Pure Chemical Industries Ltd.; product code 034-0991, or equivalent) to 30 g in 0.1 M acetate buffer (pH 5.0) in a beaker, with stirring. Prepare immediately before use. Cool the solution in a water bath during and after preparation.

Sample preparation

Dilute the sample to 0.045 - 0.230 U/ml in a glass container using Enzyme diluent solution.

Standard curve preparation

Prepare a standard curve as follows.
Set up five 100 ml volumetric flasks and label them Standards 1- 5. Dilute aliquots of the D-Glucose stock solution with deionised water as shown in the table below.

Standard No.	D-Glucose stock solution, ml	[D-Glucose] in the volumetric flask, mmol/ml	[D-Glucose] in the test tube, µmol/ml
1	1	0.1	14.3
2	2	0.2	28.6
3	4	0.4	57.1
4	6	0.6	85.7
5	8	0.8	114.3

1. Accurately measure 0.4 ml of each Standard solution into a test tube; add 0.4 ml of Phenol solution (5%; w/v) to each tube and shake thoroughly.
2. Incubate the test tubes in a water bath held at 37 °C for approximately 10 min.
3. Add 2 ml of concentrated sulfuric acid vertically to each tube using an automatic dispenser; vigorously mix the solutions using a vortex mixer.
4. Allow the solutions to stand at room temperature for approximately 30 min.
5. Record the absorbance of each solution at 490 nm against a deionised water blank.
6. Plot the absorbance of the solutions against the concentration of D-glucose (µmol/ml) in the test tube. Determine the slope and y-intercept of the standard curve.

Procedure

For each Sample preparation to be analysed, test in duplicate as follows.

1. Accurately measure 1.2 ml of the Substrate Solution into each of nine test tubes and incubate for 5 min in a 37 °C water bath.
2. Add 0.2 ml of the Sample preparation to each tube; mix and allow to react for 30 min in the 37 °C water bath.
3. After exactly 30 min, add 0.2 ml of 0.5 M hydrochloric acid to each tube to stop the reactions.
4. Transfer the solutions to individual Eppendorf tubes and centrifuge all tubes at 4 °C and 15000 rpm for 10 min.
5. Recover the supernatant from each tube using a 1 ml syringe and remove the insoluble material with Millex-LH (4 mm; 0.45 µm).
6. Accurately measure 0.4 ml of the supernatant liquid from the each of the Eppendorf tubes into a corresponding test tube; add 0.4 ml of Phenol solution (5%; w/v) to each of the nine tubes.
7. Mix the test tubes using a vortex mixer, then incubate for approximately 10 min in a 37 °C water bath.
8. Add 2 ml of sulfuric acid vertically to each tube using an automatic dispenser; vigorously mix the solutions using a vortex mixer.

9. Allow the solutions to stand at room temperature for approximately 30 min. Record the absorbance at a wavelength of 490 nm.
10. Prepare Enzyme blank solutions as follows: for each enzyme sample being tested, combine 0.2 ml of the Sample preparation and 0.2 ml of 0.5 M Hydrochloric acid in a test tube. Add 1.2 ml of the Substrate solution to the tube and follow the process above, beginning at Step 4.

Calculation

Calculate the activity of each sample in U/g as follows:

$$\beta - glucanase\ Activity, U/g = \frac{(A_1 - A_2) - b}{m} \times \frac{1.6}{C \times 0.2 \times 30}$$

Where

A_1 is the absorbance of the Reaction solution

A_2 is the absorbance of the Enzyme blank solution

b is the y-intercept of the standard curve

m is the slope of the standard curve

1.6 is the total volume of the Reaction solution (ml)

0.2 is the volume of Sample preparation in the Reaction solution (ml)

30 is the reaction time (min)

C is the concentration of enzyme in the Sample preparation (g/ml)